# Studies on the nanotubes formed by 2-phenyl-5-(4-diphenylyl)1,3,4-oxadiazole and cyclodextrins

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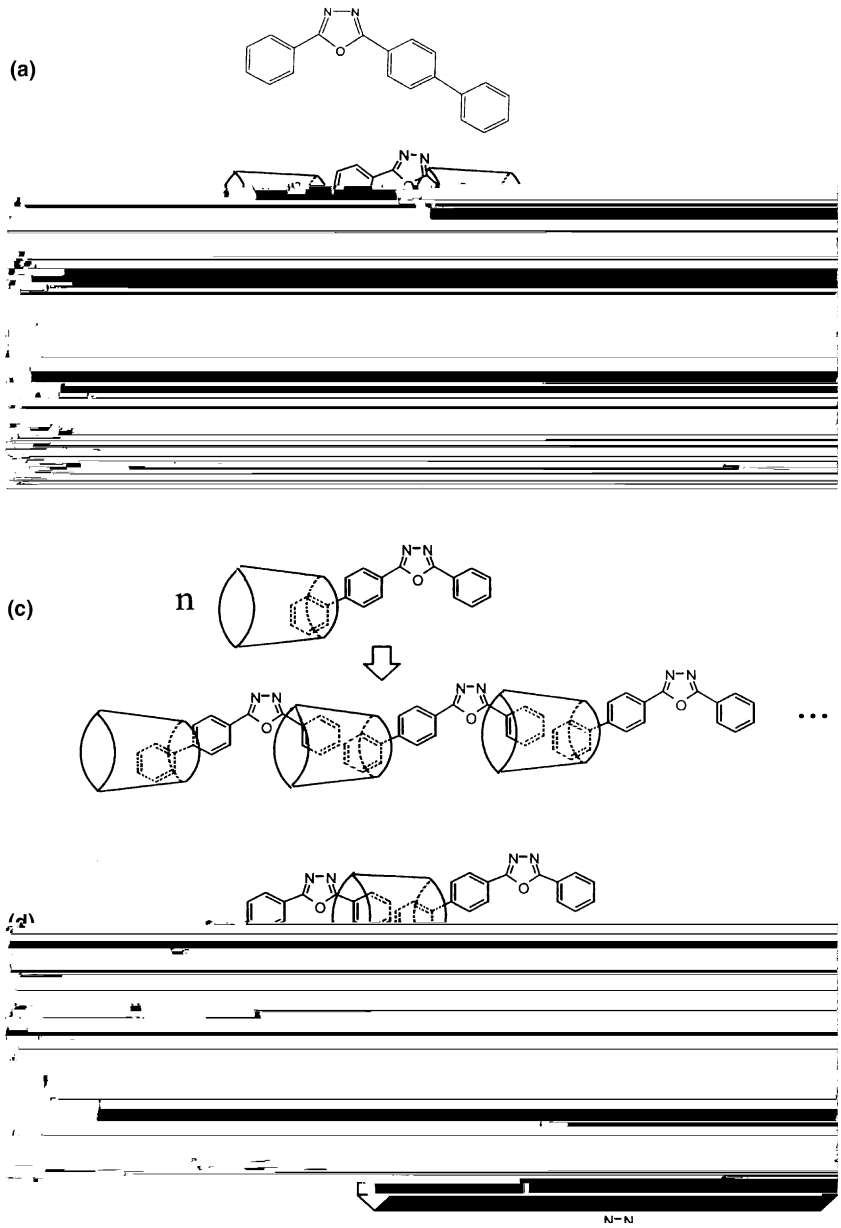


Fig. 1. The structures of PBD (a) and the 1:2 inclusion complex of PBD-α-CD (b); and the structures of nanotubes of PBD-β-CD (c), PBD-γ-CD (basic unit is 2PBD: γ-CD) (d), and PBD-γ-CD (basic unit is PBD: γ-CD) (e).

temperature-dependent liquid crystals [18]. They also inferred from the absence of excimer spectra in the β-CD solution that the nanotube cannot be formed between these oxazoles and β-CD [16,17]. However, the results reported in the text of this Letter clearly show that the PBD-β-CD nanotube can be E–ZDkwffed at relatively higher concentrations of both PBD and β-CD, while PBD exhibits monomeric fluorescence. This arises an interesting question, that is, what is the difference in the structural E–ZDeatures between the ββCDD and PBD-γ-CD nanotubes? A E–ZDurther question is

whether the picture about the structure of the PBD-γ-CD nanotube as suggested by Agbaria and Gill is reasonable. To elucidate these problem, we will study the different interaction patterns between PBD and α-, β-, and γ-CDs within relatively wide range of concentrations. We will also ascertain the average number of cyclodextrin molecules included in each nanotube. On the basis of above results, the mechanism of the formation of the nanotube can be understood better.

To the best of our knowledge, no small molecules other than the ones as mentioned above, i.e., DPH, PBD, PPD, PPO, and BBOD have been reported to form nanotube with CDs. Thus, it should be theoretically important [18,19] to study the structural features and the mechanism of the formation of the nanotubes between CDs and small molecules. This will help finding more such small molecules and exploring the potential applications of the cyclodextrin nanotube.

## 2. Experimental section

### 2.1. Materials

PBD (Acoros) and butyl-PBD (Acoros) were of scintillation grade and used without further purification. β-CD (Beijing Shuanghuan, China) was triply recrystallized from tridistilled water. α-CD (Acoros, 98%+) and γ-CD (Aldrich, 99%) were used as received. All other chemical reagents used in this study were of analytical grade. Tridistilled water was used throughout the experiments.

### 2.2. Instruments

Steady-state fluorescence and polarization measurements were performed using RF-5301 (Shimadzu) spectrofluorimeter. Both the excitation and emission bandpasses were 3 nm. The excitation and emission wavelengths were 290 and 370 nm for PBD, 300 and 370 nm for butyl-PBD, respectively.

### 2.3. Methods

Stock solutions of PBD and butyl-PBD were prepared in ethanol. For each sample, an aliquot of the stock solution was injected into the solvent, and the added volume of the stock solution was no more than 1%. The PBD-α-CD solution was prepared by adding appropriate amounts of stock solutions and α-CD to the 5 ml flask, and diluting to the mark with tridistilled water. In order to prepare the nanotube solution, the following procedures were performed: (1) an aliquot of the stock solution of PBD or butyl-PBD was added to volumetric flasks; (2) the ethanol was evaporated completely under nitrogen bubble; (3) required amounts of 10 mM CDs were added; (4) tridistilled water was finally added to give a certain volume. The above mixture was sonicated for 1 h, and then incubated for one night before carrying out any measurements. Except in the temperature experiments, all other measurements were carried out at room temperature.

In the temperature studies, sample was put in thermostat for at least 20 min to reach equilibrium, then was quickly removed to sample holder and to be measured. The whole process took no more than 1 min. In order to testify validity of this method, each sample was successively measured twice and it was found that the errors were not more than 5%. Thus, it seems possible to roughly examine the temperature effect using the above method.

The fluorescence anisotropy ($r$), measured by front-face excitation [20], can be given by

$$r = \frac{I_{0,0} - GI_{0,90}}{I_{0,0} - 2GI_{0,90}}, \tag{1}$$

where $I$ denotes the fluorescence intensity, while its first and second subscripts refer to the settings of excitation and emission polarizers, respectively. $G$ is the ratio of $I_{90,0}$–$I_{90,90}$, which is an instrumental factor reflecting the polarization characteristics of the photometric system. The error in the value of anisotropy is estimated to be as high as 0.005 in samples with very weak emission; in all other samples the errors were lower.

## 3. Results

### 3.1. The interaction of PBD with α-CD

Fig. 2a depicts fluorescence spectra of PBD ($1 \times 10^{-6}$ M) in the aqueous solutions of α-CD.

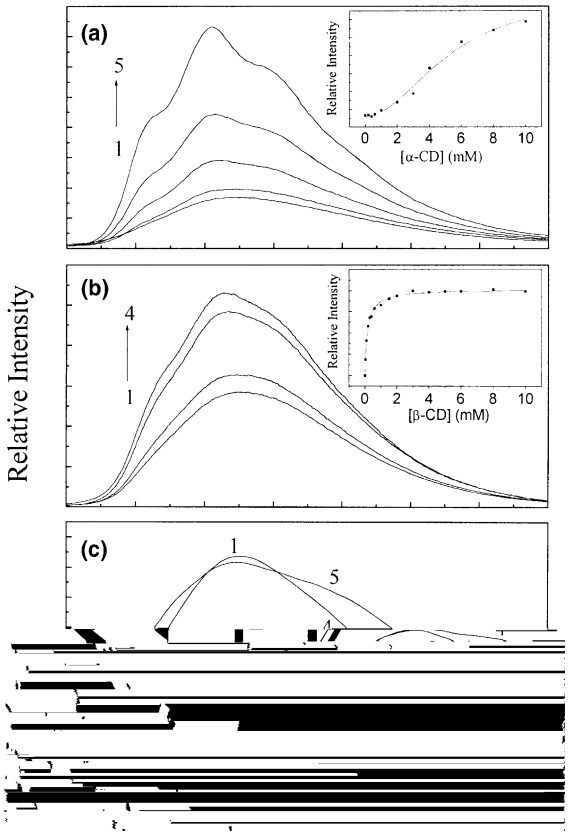


Fig. 2. Fluorescence spectra of PBD in CDs: (a) from 1 to 5, [α-CD] = 0, 0.4, 1, 2, 10 mM. The inset shows the relative fluorescence intensity vs [α-CD]; (b) from 1 to 4, [β-CD] = 0, 0.04, 1, 10 mM. The inset shows the relative fluorescence intensity vs [β-CD]; (c) from 1 to 5, [γ-CD] = 0, 0.1, 0.6, 4, 10 mM.

It is noted that the fluorescence intensity of PBD increases greatly and a small blue-shift occurs on going from water to aqueous solutions of α-CD. This suggests that PBD moves from water to a less aqueous site and in inclusion complex might be formed. To estimate the association constants and stoichiometries of the inclusion complexes, one can consider the following situations [5–8]:

**Case 1.** Only the 1:1 inclusion complex is formed,

$$I = \frac{I_0 + I_1 K_1 [\mathrm{CD}]_0}{1 + K_1 [\mathrm{CD}]_0}. \tag{2}$$

**Case 2.** 1:1 and 1:2 complexes coexist

$$I = \frac{I_0 + I_1 K_1 [\mathrm{CD}]_0 + I_2 K_1 K_2 [\mathrm{CD}]_0^2}{1 + K_1 [\mathrm{CD}]_0 + K_1 K_2 [\mathrm{CD}]_0^2}. \tag{3}$$

**Case 3.** Only the 1:2 inclusion complex is formed

$$I = \frac{I_0 + I_2 K_1 K_2 [\mathrm{CD}]_0^2}{1 + K_1 K_2 [\mathrm{CD}]_0^2}, \tag{4}$$

where $I_0$, $I_1$, and $I_2$ are fluorescence intensities of a fluorescent probe in pure water, in 1:1 and 1:2 inclusion complexes, respectively, while $K_1$ and $K_2$ denote the association constants of 1:1 and 1:2 inclusion complexes. $[\mathrm{CD}]_0$ represents the initial concentration of cyclodextrin.

Reasonable results can be obtained only when Case 3 applies. The fit based on Eq. (4) converged well with a correlation coefficient $r^2 = 0.99$ (see the inserted plot of Fig. 2a). The value of $K_2$ is estimated to be $(3.36 \pm 0.57) \times 10^4$ $\mathrm{M}^{-2}$. Studies were also performed at different concentrations of PBD. The concentrations of PBD were $2 \times 10^{-8}$, $1 \times 10^{-7}$, and $8 \times 10^{-6}$ M, respectively, and the values of association constants are $(4.28 \pm 0.79) \times 10^4$, $(2.93 \pm 0.65) \times 10^4$, and $(2.56 \pm 0.68) \times 10^4$ $\mathrm{M}^{-2}$, respectively. Moreover, it was found that the stochiometry is 1:2 at the above concentrations of PBD which leads us to conclude that each PBD can link two α-CD molecules (its structure is shown in Fig. 1b).

### 3.2. The interaction of PBD with β-CD

#### 3.2.1. Evidences for the existence of the nanotube in the PBD-β-CD solution

For the PBD-β-CD system, we also tried to calculate its association constants and stochiometries when the concentrations of PBD were $2 \times 10^{-8}, 4 \times 10^{-8}, 1 \times 10^{-7}, 1 \times 10^{-6}$, and $8 \times 10^{-6}$ M, respectively. It was found that at $[\mathrm{PBD}] \leqslant 4 \times 10^{-8}$ M, one can obtain reasonable results only when Eq. (2) is considered (see Fig. 2b and the inserted plot). This means that PBD and β-CD can form the 1:1 inclusion complex at lower concentration of PBD. The association constant $K_1$ is estimated to be $6600 \pm 420$ $\mathrm{M}^{-1}$. However, when the concentration of PBD increases, no reasonable

results can be obtained using the above three models. This suggests that some different structures might be formed at higher concentrations of PBD. So, we investigated the behavior of PBD at $1.0 \times 10^{-5}$ M in the aqueous solution of 10 mM β-CD.

The above PBD-β-CD solution is turbid, while individual solutions of β-CD (10 mM) or PBD ($1.0 \times 10^{-5}$ M) are clear, implying that some complexes in large size might exist. The similar phenomenon has also been observed for the PBD-γ-CD system, which was explained by Agbaria and Gill as the evidence for the existence of nanotube [16,17].

The $r$ value measured is 0.278 for the above system, while it is only 0.028 for PBD ($1.0 \times 10^{-5}$ M) without β-CD. The large anisotropy of PBD monomer fluorescence indicates that the rotation of PBD is largely limited in the rigid nanotube.

### 3.2.2. Relative size of the PBD-β-CD nanotube

The measurement of the steady-state fluorescence anisotropy provides a method of estimating the relative size of the PBD-β-CD nanotube according to Perrin–Weber formula [20]:

$$\frac{r_0}{r} = 1 + \frac{\tau RT}{\eta V}, \tag{5}$$

where $r_0$ is the maximum value of anisotropy for a certain probe. For PBD, the measured value of $r_0$ is 0.371 in the vitrified solution of glycerol [19]. $\tau$ is the fluorescence lifetime, while $\eta$ is the viscosity of the medium. When the fluorescence lifetime and viscosity remain constant, an increase in the fluorescence anisotropy suggests an increase in the size of the complex. In this way, the relative size of the PBD-β-CD complex could be estimated by means of the corresponding values of $r$ [19]

$$\frac{r_2(r_0 - r_1)}{r_1(r_0 - r_2)} = \frac{V_2}{V_1}, \tag{6}$$

where $r_1$ and $r_2$ are the values of the fluorescence anisotropy measured in two different systems, whereas $V_1$ and $V_2$ stand for the effective volumes of these two systems.

To estimate the number of β-CD molecules in the nanotube, the fluorescence anisotropy values ($r_2$) of PBD at varying concentrations of β-CD were investigated. If introducing the values of 1:1 PBD-β-CD complex ([PBD] = $4 \times 10^8$ M, [β-CD] = 1 mM, $r_1 = 0.056$), the value of $V_2/V_1$ is equal to the number of $\beta$-CD molecules in the nanotube [19] (Fig. 3a). The plots show that with increasing the concentration of β-CD, the fluorescence anisotropy of PBD and the number of β-CD first increase, and then reach a plateau at [β-CD] = 8 mM. This implies that most of the PBD in the solution can be included by β-CD at [β-CD] $\geqslant$ 8 mM. The maximum number of β-CD in the nanotube is estimated to be about 17.

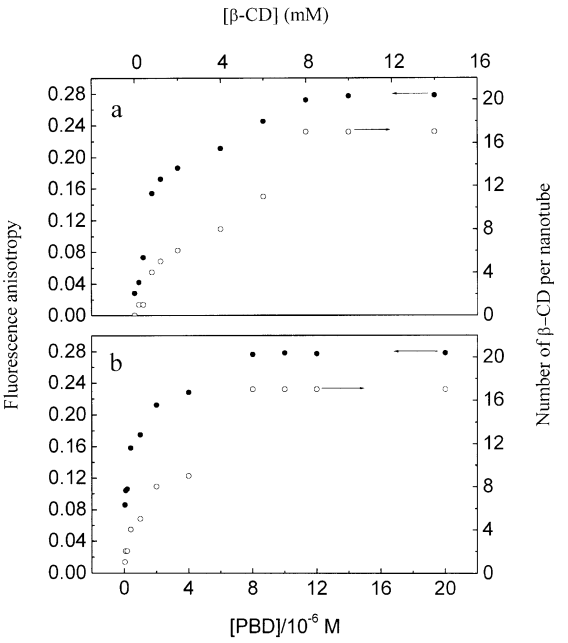


Fig. 3. Steady-state fluorescence anisotropy ($r$) and average number of β-CD per nanotube vs [β-CD] ([PBD] = $1.0 \times 10^{-5}$ M) (a) and [PBD] ([β-CD] = 10 mM) (b).

The value of the fluorescence anisotropy and the number of β-CD molecules in the nanotube at various concentrations of PBD ([β-CD] = 10 mM) are shown in Fig. 3b. It can be seen that when the concentration of PBD is $8 \times 10^6$ M, $r$ reaches a constant level.

### 3.2.3. The influence of temperature on the formation of nanotube

Some physical or chemical conditions may have important influence on the formation of the

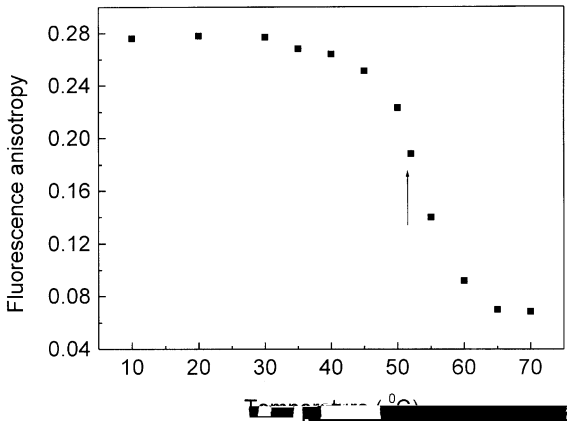


Fig. 4. Plot of the steady-state fluorescence anisotropy as a function of temperature.

nanotube. Fig. 4 displays the fluorescence anisotropy of PBD as a function of temperature. From this plot, one can learn that the fluorescence anisotropy decreases as the temperature increases, which means that the rotation of PBD becomes fast and that the nanotube is unstable at high temperature. The transition temperature where the nanotube may decompose to basic units is estimated to be around 52 °C.

### 3.3. The interaction of PBD with γ-CD

The interactions between PBD and γ-CD at higher concentrations of both PBD and γ-CD have been studied by Agbaria and his co-worker [17]. It was found that the nanotube was formed in this solution. We further measured the fluorescence anisotropy of this nanotube to be 0.291 at $[PBD] = 1 \times 10^{-5}$ M and [γ-CD] = 10 mM, and obtained the average number of γ-CD in each nanotube is about 19.

The emission spectra of PBD at lower concentration ($[PBD] = 4 \times 10^{-8}$ M) in aqueous solutions of different concentrations of γ-CD are shown in Fig. 2c. One peak at 370 nm is exhibited and a decrease in this emission band can be observed in the presence of lower concentrations of γ-CD. However, when the concentration of γ-CD is increased to a certain extent, another peak at longer wavelength occurs. Similar phenomena were observed in the γ-CD-$TFT^+$ solution [21]. Referring to the author's comment in [21], we describe the phenomena as following: the decrease in the emission intensity at lower concentration of γ-CD was ascribed to the self-quenching of the fluorescence of PBD by the inclusion of two PBD molecules into one cavity of γ-CD, and the new band at higher concentration of γ-CD results from the formation of excimer of PBD in a certain novel structure other than the 2:1 inclusion complex. In order to ascertain whether this structure is nanotube, the $r$ values were measured and the number of γ-CD ($n_{\gamma\text{-CD}}$) was calculated (see Table 1). It was seen that the nanotube can be formed at relatively higher concentration of γ-CD, although the concentration of PBD is very low. It should be pointed out that the solution in this case is still clear although the nanotube exists.

### 3.4. The interaction of butyl-PBD with CDs

The interactions between butyl-PBD and CDs were also studied. For comparison, the fluorescence spectra of PBD ($1 \times 10^{-5}$) and butyl-PBD ($1 \times 10^{-5}$ M) in different CDs (10 mM) are shown in Fig. 5, and the fluorescence anisotropy values of butyl-PBD are calculated to be 0.032, 0.109, 0.267, and 0.286 in $H_2O$, α-, β-, and γ-CDs, respectively, which indicates that the interaction pattern of butyl-PBD with CDs is similar to that of PBD with CDs. It was also found that butyl-PBD ($2 \times 10^8$ M) can form 1:1 inclusion complex with β-CD at lower concentration and the association constant was estimated to be $728 \pm 43$ $M^{-1}$ ($r^2 = 0.99$, figure not shown). Introducing the fluorescence anisotropy value of this

Table 1
Steady-state fluorescence anisotropy values ($r$) of FED ($2.0 \times 10^{-8}$ M) at different [γ-CD] and average number of γ-CD ($n_{\gamma\text{-CD}}$) per nanotube

| [γ-CD] (mM) | $r$ | $n_{\gamma\text{-CD}}$ |
|---|---|---|
| 0.04 | 0.039 | 0 |
| 0.10 | 0.060 | 1 |
| 0.40 | 0.083 | 1 |
| 0.60 | 0.112 | 2 |
| 1.00 | 0.134 | 3 |
| 2.00 | 0.157 | 4 |
| 4.00 | 0.174 | 5 |
| 6.00 | 0.193 | 6 |
| 10.0 | 0.221 | 8 |

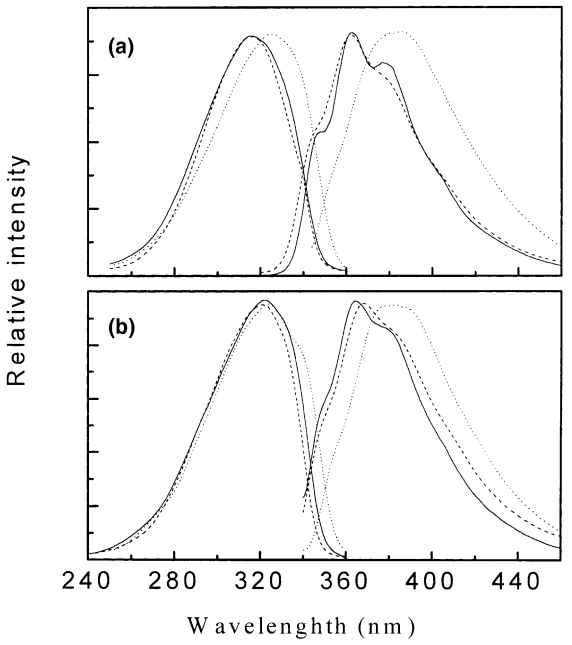


Fig. 5. Excitation and fluorescence spectra of PBD ($1 \times 10^{-5}$ M) (a) and butyl-PBD ($1 \times 10^{-5}$ M) (b) in the aqueous solutions of α-CD (10 mM, dashed line), β-CD (10 mM, continuous line), and γ-CD (10 mM, dotted line).

1:1 inclusion complex (0.058) and $r_0$ of butyl-PBD (0.368) to Eq. (6), one can obtain the number of CDs in nanotubes at higher concentration. The calculated number of butyl-PBD in the above solution of β, and γ-CD are 14 and 17, respectively.

### 3.5. Discussion

It is very interesting to note from the above results that the interaction patterns between PBD and α-, β-, and γ-CDs are much different. Within the whole ranges of concentrations of PBD and α-CD studied in this Letter, only the 1:2 inclusion complex is formed. It seems not difficult to understand that the nanotube cannot be formed when considering the relatively small cavity of α-CD and the fact that the 1:2 inclusion complex between PBD and α-CD cannot be linked together. In the case of PBD interacting with β-CD, however, the basic structural unit is the 1:1 inclusion complex. Obviously, the nanotube can be formed when the concentrations of PBD and/or β-CD are high enough for the 1:1 inclusion complex to be aggregated one by one (see Fig. 1c).

Compared with the PBD-β-CD nanotube, the PBD-γ-CD nanotube seems much easier to be formed. As can be seen from Fig. 2c and Table 1, the 2:1 inclusion complex between PBD and γ-CD can be associated to form the nanotube at very low concentration of PBD. Fig. 2e also shows that an equilibrium actually exists between the 2:1 inclusion complex and the nanotube.

On the basis of the above results and discussion, the reason why the excimer fluorescence is exhibited in the PBD-γ-CD system but not in the PBD-β-CD can be understood.

For the PBD-γ-CD system, when the 2:1 inclusion complexes are linked one by one (see Fig. 1d), the PBD molecules overlaps in the nanotube leading to the occurrence of the excimer fluorescence. Nevertheless, another possible picture for the structure of the PBD-γ-CD nanotube as shown in Fig. 1e cannot be excluded completely. In this picture, the nanotube is formed by the 'empty' γ-CD molecule and the 2:1 inclusion complex. In this case, the structural feature of the PBD-γ-CD nanotube is similar to that of the PBD-β-CD. Since the γ-CD cavity is larger than the β-CD cavity, two atomic rings of PBD are still overlapped, so that the excimer fluorescence can be observed. The above two structures of the nanotubes can be represented by -PCP-PCP-PCP-$\cdots$ (Model 1) and -PCP-C-PCP-C- $\cdots$ (Model 2), where PCP and C denote the 2:1 inclusion complex and the 'empty' γ-CD, respectively.

The above suggestion can be supported, to some extent, by the interaction of butyl-PBD with CDs. It has been found that the interaction pattern of butyl-PBD with CDs is similar to that of PBD with CDs. According to Model 1, the space of γ-CD cavity would be not large enough for the formation of the nanotube. Nevertheless, more evidence are needed to support the structural models presented here.

## 4. Conclusion

The experiments of steady-state fluorescence suggest that the interaction patterns between PBD

and α-, β-, and γ-CD are different. α-CD can form a simple inclusion complex with PBD in a stoichiometry of 1:2 (guest:host). β-CD can form 1:1 inclusion complex with PBD at lower concentration and nanotube can be formed when the concentrations of PBD and β-CD are high enough. The basic structural unit of this nanotube is 1:1 inclusion complex (see Fig. 1c). γ-CD can also form nanotube with PBD and compared with the PBD-β-CD nanotube, the PBD-γ-CD nanotube seems much easier to be formed. It was found that excimer fluorescence only exists in the solution of γ-CD, so Model 1 (see Fig. 1d) in which one pair of PBD molecules are included in one γ-CD cavity was presented. Nevertheless, another possible structure, Model 2 (see Fig. 1e), cannot be excluded completely, and this model is supported, to some extent, by the fact that the interaction pattern of butyl-PBD with CDs is similar to that of PBD with CDs.

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